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(54) Title: ENVIRONMENTALLY REGULATED GENES, INVOLVED IN THE VIRULENCE OF *STREPTOCOCCUS SUIS*

(57) Abstract: The invention relates to the field of diagnosis of and vaccination against Streptococcal infections and to the detection of virulence markers of Streptococci. The invention provides a method for modulating virulence of a *Streptococcus* comprising modifying a genomic fragment of said *Streptococcus* wherein said genomic fragment comprises at least a functional part of a fragment identifiable by hybridisation in *Streptococcus suis* to a nucleic acid or fragment thereof.

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INTERNATIONAL SEARCH REPORT

International application No

PCT/NL 02/00073

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/315 A61K39/09

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

SEQUENCE SEARCH, EMBL, EPO-Internal, BIOSIS, CHEM ABS Data, SCISEARCH, BIOTECHNOLOGY ABS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KAWABATA S ET AL: "Molecular cloning, sequence and characterization of a novel streptococcal phosphoglycerate dehydrogenase gene." ORAL MICROBIOLOGY AND IMMUNOLOGY, vol. 15, no. 1, February 2000 (2000-02), pages 58-62, XP002213084 ISSN: 0902-0055 page 59, right-hand column, paragraph 2; figure 1 --- -/-	9-11, 14



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>SMITH D ET AL: "Identification and characterization of the cps locus of Streptococcus suis serotype 2: the capsule protects against phagocytosis and is an important virulence factor"</p> <p>INFECTION AND IMMUNITY, AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, US, vol. 67, no. 4, April 1999 (1999-04), pages 1750-1756, XP002126886</p> <p>ISSN: 0019-9567</p> <p>cited in the application</p> <p>the whole document</p> <p>---</p>	
A	<p>SMITH H E ET AL: "MUTANTS OF STREPTOCOCCUS SUIS TYPES 1 AND 2 IMPAIRED IN EXPRESSION OF MURAMIDASE-RELEASED PROTEIN AND EXTRACELLULAR PROTEIN INDUCE DISEASE IN NEWBORN GERMFREE PIGS"</p> <p>INFECTION AND IMMUNITY, AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, US, vol. 64, no. 10, October 1996 (1996-10), pages 4409-4412, XP000990325</p> <p>ISSN: 0019-9567</p> <p>cited in the application</p> <p>the whole document</p> <p>---</p>	
A	<p>DATABASE EMBL 'Online!</p> <p>EBI; 7 April 1992 (1992-04-07)</p> <p>MCNAB, R.: "S. gordonii partial aldB gene, cshA gene & fbpA gene"</p> <p>Database accession no. X65164</p> <p>XP002213089</p> <p>cited in the application</p> <p>the whole document</p> <p>---</p>	
A	<p>MCNAB RODERICK: "Cloning and sequence analysis of thymidine kinase from the oral bacterium Streptococcus gordonii."</p> <p>FEMS MICROBIOLOGY LETTERS, vol. 135, no. 1, 1996, pages 103-110, XP002213085</p> <p>ISSN: 0378-1097</p> <p>the whole document</p> <p>---</p>	
A	<p>KOSKINIEMI SATU ET AL: "Identification of two genes, cpsX and cpsY, with putative regulatory function on capsule expression in group B streptococci."</p> <p>FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY, vol. 21, no. 2, June 1998 (1998-06), pages 159-168, XP002213086</p> <p>ISSN: 0928-8244</p> <p>cited in the application</p> <p>the whole document</p> <p>---</p>	

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INTERNATIONAL SEARCH REPORT

Intern al Application No

PCT/NL 02/00073

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MUNOZ ROSARIO ET AL: "Characterization of IS1515, a functional insertion sequence in Streptococcus pneumoniae." JOURNAL OF BACTERIOLOGY, vol. 180, no. 6, March 1998 (1998-03), pages 1381-1388, XP002213087 ISSN: 0021-9193 the whole document ----	
A	SEGRS RUUD P A M ET AL: "Characterisation of the gene encoding suilysin from Streptococcus suis and expression in field strains." FEMS MICROBIOLOGY LETTERS, vol. 167, no. 2, 15 October 1998 (1998-10-15), pages 255-261, XP002213088 ISSN: 0378-1097 cited in the application the whole document ----	
P,X	SMITH H E ET AL: "ENVIRONMENTALLY REGULATED GENES OF STREPTOCOCCUS SUIS: IDENTIFICATION BY THE USE OF IRON-RESTRICTED CONDITIONS IN VITRO AND BY EXPERIMENTAL INFECTION OF PIGLETS" MICROBIOLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, READING, GB, vol. 147, no. 2, February 2001 (2001-02), pages 271-280, XP000992121 ISSN: 1350-0872 table 2 -----	1-3,5-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 02/00073

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 5-18 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-3, 5-18 (all partly)

A method for modulating virulence of a Streptococcus by modifying a genomic fragment of said Streptococcus, which is identifiable by hybridisation in Streptococcus suis to the nucleic acid iri 1, 6, 22 of figure 6 (6A) and ivs 25 (Figure 6 A1 and Table 4, page 46) (SEQ ID NO's: 10 and 36). Furthermore methods, clones, isolated recombinant nucleic acids, vectors, host cells, proteins, vaccines and diagnostic tests, all related to the above nucleic acid.

Inventions 2-4: claims 1-3, 5-18 (all partly)

For groups 2-4.

A method for modulating virulence of a Streptococcus by modifying a genomic fragment of said Streptococcus, which is identifiable by hybridisation in Streptococcus suis to either the nucleic acid iri 2 and ivs 6, 7, 13, 14; iri 10, 20 and ivs 2, 4, 28; iri 24 and ivs 23, 24, respectively of figure 6 (6H, 6J1; 6B, 6X; 6J, 6Z, respectively and Table 4, pages 46-47) (SEQ ID NO's: 17 and 45; 11 and 33; 19 and 35, respectively). Furthermore methods, clones, isolated recombinant nucleic acids, vectors, host cells, proteins, vaccines and diagnostic tests, all related to the above nucleic acid.

Inventions 5-17: claims 1, 2, 5-18 (all partly)

For groups 5-17.

A method for modulating virulence of a Streptococcus by modifying a genomic fragment of said Streptococcus, which is identifiable by hybridisation in Streptococcus suis to either the nucleic acid iri 23; iri 7; iri 11; iri 14; iri 16; iri 32; iri 34; iri 13, 15, 27; iri 29; iri 18; iri 3; iri 4; iri 8, 26; respectively of figure 6 (6I; 6P; 6C; 6E; 6F; 6M; 6N; 6D; 6K; 6G; 6L; 6O; 6Q, respectively and Table 2, pages 42-43) (SEQ ID NO's: 18; 25; 12; 14; 15; 22; 23; 13; 20; 16; 21; 24; 26, respectively). Furthermore methods, clones, isolated recombinant nucleic acids, vectors, host cells, proteins, vaccines and diagnostic tests, all related to the above nucleic acid.

Inventions 18-33: claims 1, 3, 5-18 (all partly)

For groups 18-33.

A method for modulating virulence of a Streptococcus by

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

modifying a genomic fragment of said Streptococcus, which is identifiable by hybridisation in Streptococcus suis to either the nucleic acid ivs 16; ivs 20; ivs 33; ivs 5, 10, 12, 22; ivs 18; ivs 3; ivs 8; ivs 1; ivs 32, 35; ivs 9, 17; ivs 11; ivs 15; ivs 29; ivs 34; ivs 36; ivs 19; respectively of figure 6 (6U; 6Y; 6F1; 6I1; 6V; 6C1; 6K1; 6R; 6E1; 6L1; 6S; 6T; 6B1; 6G1; 6H1; 6W, respectively and Table 4, pages 46-47) (SEQ ID NO's: 30; 34; 41; 44; 31; 38; 46; 27; 40; 47; 28; 29; 37; 42; 43; 32, respectively). Furthermore methods, clones, isolated recombinant nucleic acids, vectors, host cells, proteins, vaccines and diagnostic tests, all related to the above nucleic acid.

Invention 34: claims 1, 3,
5-18 (all partly) and 4 (completely)

A method for modulating virulence of a Streptococcus by modifying a genomic fragment of said Streptococcus, which is identifiable by hybridisation in Streptococcus suis to the nucleic acid ivs 31 of figure 6 (6D1 and Table 4, page 46) (SEQ ID NO: 39) and which genomic fragment comprises a gene encoding a fibronectin/fibrinogen binding protein. Furthermore methods, clones, isolated recombinant nucleic acids, vectors, host cells, proteins, vaccines and diagnostic tests, all related to the above nucleic acid.